

### ***Remarks***

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 25, 29-33, 36, 42 and 43 are pending in the application, with claims 25 and 36 being the independent claims. Claims 26-28, 34-35 and 37-41 are sought to be cancelled without prejudice to or disclaimer of the subject matter therein. The amendments to claims 25 and 36 are based, inter alia, on previously presented Claims 32 and 43, so no new matter is added. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

### ***Description of the Invention***

The Examiner states that is not precisely clear where the figures are described in the specification.

Applicants have amended the specification to include a Brief Description of the Figures, as requested by the Examiner.

### ***Objections to the Drawings***

The Examiner objected to Figure 1 because it is not clear from the figure or the specification what is being measured. The Examiner asserts that it is not clear how absorbance is related to the variable of oxidative protection. Applicants respectfully disagree.

Applicants believe that no correction to Figure 1 is required. First, it is believed that the Examiner has erroneously referred to Figure 1, rather than Figure 5, which shows experimental results. Specifically, duroquinone is known to induce oxidative stress in mitochondria. In the assay, absorbance at 570nm is in proportion to mitochondrial respiratory chain activity. Greater activity results in a greater amount of conversion of MTT to formazine dye crystals and hence greater absorbance. Put otherwise, a higher absorbance indicates healthier mitochondria. When there is no duroquinone (black triangle), the mitochondria remain healthy. When there is 50mM duroquinone and no added agent of the invention (open square), the absorbance is reduced compared to that of untreated, healthy mitochondria. Further, it is known that presence of potassium ions is required to mimic neuronal stimulation *in vivo*, as potassium ions are needed to recycle the toxin receptor. Therefore, in the assay, an agent of the invention is added both in the absence and presence of potassium ions. It is seen from the figure that in the absence of potassium ions (black square) the mitochondrial activity remains low, i.e. the mitochondria are not protected from the effects of duroquinone. It is also seen that in the presence of potassium ions (black diamond) the absorbance increases with increasing concentration of the agent of the invention and the mitochondria are therefore protected by the agent of the invention, in the presence of potassium ions, against the oxidative stress caused by duroquinone. Therefore, when Figure 5 is read in combination with Example 10, it is clear what activities are being measured. Withdrawal of this objection is respectfully requested.

***Sequence Listing***

According to the Examiner, the present application is not fully in compliance with the Sequence Rules.

A substitute Sequence Listing is being submitted concurrently with this Amendment and Reply.

In accordance with 37 C.F.R. § 1.821(g), this submission includes no new matter.

In accordance with 37 C.F.R. § 1.821(f), the paper copy of the Sequence Listing and the computer readable copy of the Sequence Listing submitted herewith in the above application are the same.

The sequence at page 10, line 22 has been included, as have the two sequences found in Figure 1. Also submitted herewith is a marked up copy of Figure 1 showing the insertion of the sequence identifiers in red. Concerning the addition of sequence identifiers throughout the specification, and specifically at page 13, it is submitted that, since there are no specific sequences set forth, it is not necessary to supply a SEQ ID NO. (See, the MPEP at 2422.03, where it is stated: "In those instances in which prior art sequences are only referred to in a given application by name and a publication or accession reference, they need not be included as part of the Sequence Listing. . . ."). Withdrawal of this objection is respectfully requested.

***Rejections under 35 U.S.C. § 112***

The Examiner rejected claims 25, 29-33, 42 and 43 under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most

nearly connected, to make and/or use the invention. Applicants respectfully traverse this rejection.

The Examiner states that the disclosure is non-enabling for use of the composition to translocate SOD into neuronal cells and reduce oxidative stress. Specifically, the Examiner states that the methods were not described in sufficient detail to enable one to determine the protective effects of the SOD composition on oxidative stress in neuronal cells. The Examiner asserts that it is not known how absorbance of light at 570 nm is related to oxidative stress, if the SOD composition was translocated into cells, if NG-108 cells are neuronal cells, or if they have receptors for Clostridium toxins. The Examiner also states that the treatment groups appear to be indistinguishable from each other and that there appears to be no concentration effect of superoxide dismutase on the measured variable. The Examiner further states that limitations for delivery of a therapeutic agent by the recited composition is not enabled.

Applicants respectfully disagree that the claims and the specification are not enabled. As stated *supra*, Figure 5 and example 10 demonstrate the activity of compositions of the invention. Duroquinone is known to induce oxidative stress in mitochondria, as shown in the abstracts of papers by Wilde *et al.*, which are attached as Exhibits A-B. In the assay described in the specification, absorbance at 570nm is in proportion to mitochondrial respiratory chain activity. Greater activity results in a greater absorbance. With no duroquinone exposure, mitochondria remain healthy. In the presence of duroquinone only, the absorbance is reduced. The agent of the invention is added both in the absence and presence of potassium ions because presence of potassium

ions is required to mimic neuronal stimulation *in vivo*. In the absence of potassium ions, the mitochondria are not protected from the effects of duroquinone. In the presence of potassium ions, the absorbance increases with increasing concentration of the agent of the invention and the mitochondria are therefore protected by the agent of the invention against the oxidative stress caused by duroquinone.

Regarding NG-108 cells, these are neuroblastoma cells, which are neuronal cells in the sense that they have receptors for clostridial neurotoxins. To demonstrate this, abstracts of papers by Yokosawa *et al.*, are attached (Exhibits C-D), confirming that the NG-108 cells are neuronal cells and that clostridial neurotoxin binds to these cells.

Concerning the Examiner's concern that the treatment groups are indistinguishable, it is shown that the composition must be translocated into the cell to have an effect. Figure 5 and Example 10 demonstrate that there was an effect of administering the composition of the invention in the presence of potassium ions, therefore the SOD effector must have been translocated into the neuronal cells.

Therefore, it is demonstrated that the cells used are neuronal cells, that the cells have a receptor for clostridial toxin, and that the composition of the invention was translocated into these cells.

The Examiner further states that the specification is not enabling for the limitations of the claims wherein the recited composition of superoxide dismutase is used to deliver a *therapeutic* agent to neuronal cells, or used as a *pharmaceutical* composition for reducing oxidative damage in neuronal cells.

Without acquiescing to the correctness of the Examiner's rejection, and in the interest of expediting prosecution, Applicants have amended claims 33 and 36 to delete the terms "pharmaceutical" and "therapeutic".

Applicants believe, therefore, that the enablement rejection is rendered moot, and respectfully request that this rejection be withdrawn.

The Examiner also rejected claims 30 and 31 under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification.

Without acquiescing to the correctness of the Examiner's rejection, and in the interest of expediting prosecution, Applicants have amended claims 30 and 31 to delete reference to fragments, variants and derivatives of neuronal cell binding domains of clostridial toxins and fragments, variants and derivatives of domains of clostridial neurotoxins that translocate polypeptide sequences into cells. Applicants therefore believe that this rejection has been obviated, and respectfully request the rejection be withdrawn.

***Rejections under 35 U.S.C. § 102***

The Examiner rejected claims 25 and 36 under 35 U.S.C. § 102(b) as being anticipated by Figueiredo *et al.* (*Exp. Neurol.*, 145: 546-554 (1997), hereinafter "Figueiredo"). Applicants respectfully traverse this rejection.

The Examiner states that Figueiredo discloses a superoxide dismutase/tetanus toxin composition which is indistinguishable from the composition of the present invention.

Applicants have amended the claims to recite that the cleavable linker is a disulfide bridge or a site for a protease found in neuronal cells. In contrast, Figueiredo does not disclose a cleavable linker which is a disulfide bridge or a site for a protease found in neuronal cells. Figueiredo merely discloses compositions where SOD is covalently linked to a toxin component. The use of the cleavable linker enables the SOD to be delivered to the intended site and, once there, to function efficiently to protect against oxidative stress. Figueiredo does not disclose a composition where a cleavable linker is employed. Since each and every aspect of the claims is not disclosed by Figueiredo, this reference cannot anticipate claims 25 and 36. Applicants believe that the amendment renders the rejection moot, and respectfully request that this rejection be withdrawn.

The Examiner also rejected claims 25 and 36 under 35 U.S.C. § 102(b) as being anticipated by Francis *et al.* (*J. Biol. Chem.*, 270(25): 15434-15442 (1995), hereinafter "Francis"). Applicants respectfully traverse this rejection.

Applicants have amended the claims to recite that the cleavable linker is a disulfide bridge or a site for a protease found in neuronal cells. In contrast, Francis does not disclose a linker which is a disulfide bridge or a site for a protease found in neuronal cells. Francis merely discloses compositions where SOD is covalently linked to a toxin component. The use of the cleavable linker enables the SOD to be delivered to the intended site and, once there, to function efficiently to protect against oxidative stress. Francis does not disclose a composition where a cleavable linker is employed. Since each and every aspect of the claims is not disclosed by Francis, this reference cannot

anticipate claims 25 and 36. Applicants believe that the amendment renders the rejection moot, and respectfully request that this rejection be withdrawn.

***Conclusion***

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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